REMARKS

Enclosed is a check for the requisite fee for a three-month extension of time. Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 06-1050. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 1-49, 51-55, 58-60, 63-76, 86, 88-124 and 127-147 are pending. Claims 1, 75 and 124 are amended herein. Claim 55 is cancelled without prejudice or disclaimer. Claim 1 is amended to more distinctly claim the subject matter. Basis for the amendment is found throughout the specification (for example, see page 22, lines 16-18). Claims 75 and 124 are amended for clarity. Basis for the amendment is found throughout the specification (*e.g.*, see page 40, lines 3-7). Claims 145-147 are added herein. Basis for new claim 145 is found throughout the specification (*e.g.*, see page 25, lines 26-30). Basis for new claims 146 and 147 is found throughout the specification (*e.g.*, see page 40, lines 3-7). Therefore, no new matter is added.

THE REJECTIONS OF CLAIMS 1-55, 58-60, 63-76, 88-124 AND 127-144 UNDER 35 U.S.C. §103(a)

Claims 1-17, 19-27, 29-33, 35-37, 43-52, 54, 64-70, 73-76, 124 and 127

Claims 1-17, 19-27, 29-33, 35-37, 43-52, 54, 64-70, 73-76, 124 and 127 are rejected under 35 U.S.C. §103(a) over Köster (WO 94/16101) in view of Cantor (US 5,503,980) because Köster allegedly teaches every element of the claims except probes comprising a single-stranded variable region or an array having a collection of probes with sufficient sequence diversity in the variable regions to hybridize all of the target sequence with complete or nearly complete discrimination, but Cantor allegedly cures these deficiencies.

In particular, the Examiner urges that Köster teaches a method for sequencing a target nucleic acid molecule, comprising:

providing a set of nucleic acid fragments each containing a sequence that corresponds to a portion of the target;

hybridizing the set to an array of nucleic acid probes, where each probe comprises a single stranded portions (referencing page 14, lines 31-33 of Köster);

where the array comprises a collection of probes with sufficient sequence diversity in the variable regions to hybridize to all of the fragments;

determining the molecular weights of nucleic acids in the target array to identify hybridized probes;

based upon the hybridized probes, determining the sequence of the target (reference page 15, lines 2-4). The Examiner then points to pages that allegedly teach limitations in dependent claims. The Examiner urges that Cantor teaches a probe with a single-stranded variable region and an array of probes that that have sufficient sequence diversity to hybridize to all of that target fragments. The Examiner concludes that it would have been obvious to one of ordinary skill in the art to have use the array of Cantor to for sequencing nucleic acids as taught by Köster. This rejection respectfully is traversed.

RELEVANT LAW

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. § 103(a), there must be (1) some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (*ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)); and (2) the combination of the cited references must actually teach or suggest the claimed invention.

Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. *Ex parte Gerlach*, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art." *In re Keller*, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (*ACS Hosp. Systems, Inc. v Montefiore Hosp.*, 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" *W.L. Gore & Associates, Inc. v. Garlock Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

THE CLAIMS

Claim 1 is directed to a method for sequencing a target nucleic acid that includes as steps fragmenting the target nucleic acid to produce a set of nucleic acid fragments each containing a sequence that corresponds to a sequence of the target nucleic acid; hybridizing the set of nucleic acid fragments to an array of nucleic acid probes to form a target array of nucleic acids, where each probe includes a single-stranded portion including a variable region such that each member of the set hybridizes to a member of the array of probes; and the array includes a collection of probes with sufficient sequence diversity in the variable regions to

hybridize all of the target sequence with complete or nearly complete discrimination; determining molecular weights of the nucleic acids in the target array to identify the hybridized probes; and based upon the hybridized probes, determining the sequence of the target nucleic acid. Claims 2-17, 19-27, 29-33, 35-37, 43-52, 54, 64-70 and 73-76 ultimately depend from claim 1 and are directed to various embodiments thereof.

Claim 124 is directed to an array of nucleic acid probes, where each probe includes a single-stranded portion and a constant double-stranded portion; each single-stranded portion includes a variable sequence; the array of probes has sufficient sequence diversity in the variable regions to hybridize to all of a target nucleic acid molecule with complete or nearly complete discrimination; the array is attached to a solid support including a matrix chemical that facilitates the volatilization of nucleic acids; and the array includes a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between at least two nucleic acid molecules when detected by mass spectrometry. Claim 127 is directed to a system that includes a mass spectrometer, a computer and the array of claim 124.

Teachings of the cited art and differences from the claims

Köster (WO 94/16101)

Köster teaches the use of mass spectrometry to analyzed the Sanger sequencing reaction mixtures. In Sanger sequencing, four families of chain-terminated fragments are obtained. The mass difference per nucleotide addition is known and is unique for each of the four nucleotides so that the mass difference between two sequential fragments is indicative of the identity of the added nucleotide. In the methods taught in Köster, the DNA sequence of a target molecule can be determined through the separate determination of the four base-specifically terminated families, and the sequence assigned via interpolation of the molecular weight peaks of the four specifically terminated families. In other embodiments, the four fragment families can be determined simultaneously. Comparison of the mass differences between fragments with the known masses of the chain terminating nucleotide allows assignment of the sequence. The differences in molecular weights among the chain terminating nucleotides can be enhanced using mass modification to increase the resolution. The mass spectra of the fragments are compared and aligned by increasing molecular weight in order to determine the nucleotide sequence from the mass spectra.

Köster teaches that the nested Sanger fragments can be immobilized to permit conditioning of the fragments by capturing them on a solid support by providing an array of linkers L' that specifically interact with L to form a photocleavable bond (see Figure 1 in

Köster). The linking functionality L is included on the sequencing primer so that the resulting extended primer can be immobilized after synthesis of the nested fragments to permit conditioning prior to mass spectrometric analysis. There is no hybridization step, nor are there probes that include a single-stranded portion comprising a variable region such that each member of the set hybridizes to a member of the array of probes; and the array comprises a collection of probes with sufficient sequence diversity in the variable regions to hybridize all of the target sequence with complete or nearly complete discrimination. In the method of Köster, the extended primers with linker are produced and then are linked to a solid support for conditioning. The immobilized fragments are not captured by probes that contain a variable region. Capture is effected via the linkers, which form a photocleavable bond. Hence Köster has virtually nothing to do with the claimed method.

Cantor (U.S. Patent 5,503,980)

Cantor teaches positional sequencing by hybridization using an array of probes in which the probes have a double-stranded portion, a single-stranded portion, and a random sequence within the single-stranded portion (col. 5, lines 40-45). Cantor teaches a method for determining a nucleotide sequence by positional hybridization (col. 7, lines 63 through col. 8, line 6). Cantor teaches determining a target nucleotide sequence by analyzing the hybridization pattern of target nucleic acid fragments on a hybridization chip, which provides a fingerprint identification of the target nucleotide sequence (col. 7, lines 6-10). Hybrids are detected by labeling; based upon the probes to which the target hybridizes, the sequence of the target molecule is determined. Cantor does not teach or suggest detecting the hybrids based upon molecular weight, including by mass spectrometry.

ANALYSIS

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

The combination of the teachings of Köster with the teachings of Cantor does not result in the instantly claimed methods, arrays or systems.

1. Claims 1-17, 19-27, 29-33, 35-37, 43-52, 54, 64-70 and 73-76

Claim 1 and dependent claims require identifying hybridized probes based upon molecular weight. Köster teaches using base-specific chain termination (Sanger sequencing) to generate a set of nested fragments of a target nucleic acid and using mass spectrometry to analyze the nested fragments via their different molecular masses. Köster teaches that comparison of the mass difference measured between the nested fragments with the known

masses of each chain-terminating nucleotide allows the sequence of each fragment to be determined. Based upon the mass of the fragments, the fragments are aligned and the sequences are determined.

In the general nucleic acid sequencing methods disclosed in Köster, the molecular weights of a series of nucleic acid fragments of different lengths, yet all subsequences of a single larger sequence, are determined by mass spectrometry. The fragments are identical throughout the portions in which they are the same length. Thus, there is redundancy in the information that is provided by the Sanger method. In addition, the very process of the Sanger reactions aids in amplifying the amount of nucleic acid to be analyzed, thereby making sensitivity of the analysis method somewhat less critical.

The fragments are compared to each other and aligned by increasing molecular weight in order to determine the nucleotide sequence. Comparison of the mass difference measured between fragments with the known masses of each chain terminating nucleotide allows the assignment of sequence to be performed. The process of Sanger sequencing is thus a comparative analysis of recurring information. Because there are built-in molecular weight reference points all along the sequencing process, there are continual "self-checks" in the comparisons of the fragments there is some room for error in molecular weight measurement. If the calculated difference is slightly "off," it is still likely that the correct nucleotide will be identified due to the differences in the molecular weights of the four separate nucleotides. Köster does not suggest detection of a single molecule and determining its molecular weight.

Furthermore, Köster does not teach or suggest fragmenting a target nucleic acid molecule and hybridizing it to probes. In the capture method noted by the Examiner, a linker is attached to the primer for Sanger sequencing. After producing the nested fragments from the primer, each fragment has a linker L attached. The linker can then be captured by a solid support that contains a complementary linker. The solid support contains linkers L'. The linkers can be complementary single strands of nucleic acid or chemical moieties that react. The L-L' linkage, however, is photolabile and is intended to be temporary. Capture is effected for purification or conditioning of the single stranded nested fragments, whose molecular weights are determined by mass spectrometry. The method does not include detection of hybrids nor deduction of a sequence based upon probes to which a target hybridizes.

In contrast, the instantly claimed method does not rely on Sanger sequencing but rather in detecting hybridized probes based upon their molecular weights. In the instantly

claimed methods, a target nucleic acid molecule is fragmented to produce a set of nucleic acid fragments each having a portion of the target nucleic acid. For example, the specification teaches that the set of target nucleic acid fragments can be produced by fragmenting the target nucleic acid into a plurality of fragments using physical, chemical or enzymatic means to create a set of fragments (*e.g.*, see page 22, lines 16-15). These fragments are hybridized to an array of nucleic acid probes. The molecular weight of the probes is measured to identify those that have formed hybrids. Hence the method is very different from the Sanger sequencing method of Köster in which sets of nested fragments are produced and the relative molecular weights of the fragments determined by mass spectrometry. Köster does not teach or suggest a method of sequencing hybridization nor does Köster teach or suggest detecting hybridized probes in an array based upon molecular weight. Thus, Köster does not teach or suggest determining the sequence of a target nucleic acid by identifying hybridized probes in the array by determining the molecular weight of the hybridized probes, and determining the sequence of the target nucleic acid by identifying the hybridized probes. Köster does not teach or suggest identifying hybridized probes, but determining a sequence based upon the differences in molecules weights among nested fragments. This method has nothing to do with sequencing by hybridization nor detecting hybridized probes based upon molecular weight.

Cantor does not cure this deficiency . Cantor does teach a method in which a target nucleic acid molecule is fragmented and labeled and its sequence determined based upon the probes to which the fragments hybridize and the location of label. The sequence is determined based upon the probes to which the target fragments hybridize. Cantor, however, does not teach or suggest detection of the hybridized probes by molecular weight. The method of Cantor relies upon labeling the target nucleic acid for detection. There is no teaching or suggestion in Cantor of identifying hybridized probes in an array by their molecular weights.

The only teachings directed to molecular weight in Cantor are the teaching in Example 2 directed to fractionation of a sample, and reference to the Maxim and Gilbert sequencing technique, where terminally labeled DNA molecules are chemically cleaved at single base repetitions and then the molecular weight of each partially cleaved fragment is determined using electrophoresis to produce a pattern of fragments on a gel, whereby the DNA sequence can be read (see col. 1, lines 24-35). Hence, Cantor does not teach or suggest identifying hybridized probes in the array by determining the molecular weight of the hybridized probes, and

determining the sequence of the target nucleic acid by identifying the hybridized probes. Hence, Cantor does not teach or suggest the subject matter missing from Köster.

Thus, combining the teachings of Köster and Cantor does not result in a method for sequencing a target nucleic acid that includes as elements fragmenting a target nucleic acid and hybridizing the fragments to an array of nucleic acid probes, identifying hybridized probes in the array by determining the molecular weight of the hybridized probes, and based on the hybridized probes, determining the sequence of the target nucleic acid. Hence, the combination of Köster and Cantor does not teach or suggest every element of claim 1. Claims 2-17, 19-27, 29-33, 35-37, 43-52, 54, 64-70 and 73-76 ultimately depend from claim 1 and include the limitations thereof. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness for claims 1-17, 19-27, 29-33, 35-37, 43-52, 54, 64-70 and 73-76.

2. Claims 124 and 127

The array of claim 124 includes as elements that each probe includes a single-stranded portion and a constant double-stranded portion and that the array includes a collection of probes with sufficient sequence diversity in the variable region to hybridize to all of the target nucleic acid molecule The array of claim 124 also includes as an element that the array includes a nucleic acid probe **having at least one mass-modifying functionality** that increases the discrimination between at least two nucleic acid molecules when detected by mass spectrometry.

The Examiner alleges that Köster teaches every element of the array of claim 124, except that the array comprises a collection of probes with sufficient sequence diversity in the variable regions to hybridize all of the target sequence with complete or nearly complete discrimination, but alleges that Cantor cures this defect.

Köster does not teach or suggest an array of nucleic acid probes, where each probe includes a single-stranded portion and a constant double-stranded portion, or an array of probes having sufficient sequence diversity in the variable regions to hybridize to all of a target nucleic acid molecule with complete or nearly complete discrimination. As noted above, Köster teaches using a solid support to capture the single-stranded nested fragments produced in the Sanger sequencing reaction. In that embodiment in which the linkers L and L' are complementary oligonucleotides, the solid support includes an array of single-stranded identical probes that capture the oligonucleotide linker on the nested fragments. The resulting array contains an array of identical double-stranded portions linked to the nested fragments. There are no random sequences nor variable regions to permit capture of any target sequence.

The single-stranded portions are the nested fragments produced in the Sanger sequencing reaction. Hence, Köster does not teach or suggest any elements of the rejected claims.

Cantor does teach an array of partially double-stranded probes that contain a single stranded region and a variable region, but Cantor does not teach or suggest an array that includes a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between at least two nucleic acid molecules when detected by mass spectrometry. Further, Cantor does not teach or suggest including matrix chemical in the array.

There is no teaching nor suggestion in Cantor of analysis of the probes by mass spectrometry, nor of including in an array a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between at least two nucleic acid molecules when detected by mass spectrometry. Thus, combining the teachings of Köster and Cantor does not result in an array of nucleic acid probes as instantly claimed. Hence, the combination of Köster and Cantor does not teach or suggest every element of claim 124.

Claim 127 recites a system that includes a mass spectrometer, a computer and the array of claim 124. Hence, the combination of the teachings of Köster and Cantor does not result in the system of claim 127. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness for claims 124 and 127.

Claim 28

Claim 28 is rejected under 35 U.S.C. §103(a) over Köster and Cantor in view of Weiss (U.S. 6,025,193) because the combination of Köster and Cantor allegedly teaches all elements of claim 28 except generation of thiol moieties by using Beucage reagent, but Weiss allegedly cures this defect. This rejection is respectfully traversed.

RELEVANT LAW

See related section above.

Claim 28

Claim 28 depends from claim 1, and is directed to an embodiment thereof where the array includes nucleic acid probes having as a mass-modifying functionality a thiol moiety that is generated by using Beucage reagent.

Teachings of the cited art and differences from the claim

Köster (WO 94/16101)

See related section above.

Cantor (U.S. Patent 5,503,980)

See related section above.

Weiss (U.S. 6,025,193)

Weiss teaches methods and compositions for diagnosing and treating pathological conditions related to a dopamine receptor abnormality, which includes administering a plasmid encoding an oligonucleotide anti-sense to one or more RNA molecules encoding one of the several dopamine receptors. The reference teaches that unmodified oligodeoxy-nucleotides can be converted into phosphorothioate oligodeoxynucleotides using standard phosphoramidite protocols but replacing the standard oxidation by iodine with Beucage reagent for sulfurization. Weiss teaches that using Beucage reagent results in the replacement of every oxygen group of the phosphodiester bond with a sulfur group, and that such substitutions result in an asymmetric distribution of the negative charge to predominate on the sulfur atom, resulting in "improved stability to nucleases, retention of solubility in water and stability to base-catalyzed hydrolysis" (col. 13, lines 2-14), improved distribution and *in vivo* stability (col. 15, lines 41-45), and activation of RNAse H (col. 13, lines 45-47). Weiss does not teach or suggest detection of hybridized probes in the method of Cantor by molecular weight.

Analysis

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

The combination of the teachings of Köster and Cantor with the teachings of Weiss does not result in the instantly claimed methods.

As discussed above, the combination of the teachings of Köster and Cantor does not teach or suggest a method for sequencing a target nucleic acid that includes as an element identifying hybridized probes in the array by determining the molecular weight of the hybridized probes, and based on the hybridized probes, determining the sequence of the target nucleic acid. Weiss does not teach or suggest the subject matter missing from the combination of the teachings of Köster and Cantor. Weiss does not teach or suggest a method for sequencing a target nucleic acid. Weiss does not teach or suggest identifying hybridized probes in an array by determining the molecular weight of the hybridized probes, and based on the hybridized probes, determining the sequence of the target nucleic acid. Thus, even if Weiss teaches generating thiol moieties using Beucage reagent, Weiss fails to cure the deficiencies in the teachings of the combination of Köster and Cantor because Weiss does not teach or suggest the elements of the claimed subject matter missing from the combination of the teachings of Köster and Cantor.

None of Köster, Cantor nor Weiss, individually nor in any combination, teaches or suggests a method for sequencing a target nucleic acid that includes as an element identifying hybridized probes in an array by determining the molecular weight of the hybridized probes, and based on the hybridized probes, determining the sequence of the target nucleic acid. Thus, combining the teachings of Köster and Cantor with the teachings of Weiss does not result in the instantly claimed method of claim 28. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

Claim 34

Claim 34 is rejected under 35 U.S.C. §103 as being unpatentable over Köster (WO 94/16101) in view of Cantor (U.S. Patent 5,503,980) because Köster allegedly teaches all elements of claim 34, except ligating the hybridized target nucleic acids to the probes, but Cantor allegedly cures this defect. This rejection is respectfully traversed.

RELEVANT LAW

See related section above.

Claim 34

Claim 34 depends from claim 1, and is directed to an embodiment thereof further including the step of ligating the hybridized target nucleic acids to the probes.

Teachings of the cited art and differences from the claimed method

Köster (WO 94/16101)
See related section above.

Cantor (U.S. Patent 5,503,980)
See related section above.

Analysis

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

The combination of the teachings of Köster with the teachings of Cantor does not result in the instantly claimed methods.

As discussed above, the combination of the teachings of Köster and Cantor does not teach or suggest a method for sequencing a target nucleic acid as recited in claim 1. Claim 34 depends from claim 1 and includes all the limitations thereof. Thus, the combination of the teachings of Köster and Cantor does not teach or suggest every element of the method of claim 34. Thus, even if Cantor teaches ligating the hybridized target nucleic acids to the probes, combining the teachings of Köster and Cantor, as discussed in detail above, does not result in a method for sequencing a target nucleic acid that includes as an element identifying

hybridized probes in the array by determining the molecular weight of the hybridized probes, and based on the hybridized probes, determining the sequence of the target nucleic acid. Hence, the combination of Köster and Cantor does not teach or suggest every element of claim 34. Therefore, the Examiner has failed to set forth a prima facie case of obviousness.

Claims 71 and 72

Claims 71 and 72 are rejected under 35 U.S.C. §103 as being unpatentable over Köster and Cantor in view of Sanghvi *et al.* (U.S. Patent No. 6,214,551) because the combination of the teachings of Köster and Cantor allegedly teaches all elements of the claims except that the selectively releasable bond is 4,4'-dimethoxy-trityl or a derivative thereof, and Sanghvi *et al.* allegedly cures this defect. The Examiner contends that Sanghvi *et al.* teaches the selectively releasable bond 4,4'-dimethoxytrityl or a derivative thereof, and argues that although the reference does not teach the derivative 3 or 4 [bis-(4-methoxy-phenyl)]-methyl-benzoic acid in particular, Sanghvi *et al.* teaches equivalent compounds and derivatives used for the same purpose. This rejection is respectfully traversed.

The claims

Claims 71 and 72 ultimately depend from claim 1 and are directed to embodiments thereof. Claim 71 is directed to the embodiment where each probe is attached to the solid support by a selectively releasable bond that includes 4, 4'-dimethoxytrityl or a derivative thereof. Claim 72 is directed to the embodiment where the derivative of 4, 4'-dimethoxytrityl is selected from the group consisting of 3 or 4 [bis-(4-methoxyphenyl)]-methyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxy-phenyl)]-methyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxyphenyl)]-hydroxy-methyl-benzoic acid, N-succinimidyl-3 or 4 [bis-(4-methoxy-phenyl)]-chloromethyl-benzoic acid and salts thereof.

Relevant law

See related section above.

Teachings of the cited art and differences from the claimed methods

Köster (WO 94/16101)

See related section above.

Cantor (U.S. Patent 5,503,980)

See related section above

Sanghvi *et al.* (U.S. Patent 6,214,551)

Sanghvi *et al.* teaches compounds that mimic and/or modulate the activity of wild-type nucleic acids. The compounds taught by Sanghvi *et al.* contain a selected nucleotide sequence

where the nucleotides are covalently bound through linking groups that contain adjacent nitrogen atoms. Sanghvi *et al.* teaches the use of dimethoxytrityl groups as a blocking group during nucleoside polymerization. Sanghvi *et al.* teaches that an oligonucleotide is tethered to a solid support via its 3' hydroxyl group (col. 57, line 63 through col. 58, line 14).

Analysis

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

The combination of the teachings of Köster and Cantor with the teachings of Sanghvi *et al.* does not result in the instantly claimed methods.

As discussed above, the combination of the teachings of Köster and Cantor does not teach or suggest methods for sequencing a target nucleic acid that include as an element identifying hybridized probes in the array by determining the molecular weight of the hybridized probes, and based on the hybridized probes, determining the sequence of the target nucleic acid. Sanghvi *et al.* does not cure this defect. Sanghvi *et al.* does not teach or suggest determining the sequence of a target nucleic acid. Sanghvi *et al.* does not teach or suggest sequencing a nucleic acid by hybridizing fragmented target nucleic acid to an array as claimed and identifying hybridized probes in the array by determining the molecular weight of the hybridized probes, and based on the hybridized probes, determining the sequence of the target nucleic acid. Hence, Sanghvi *et al.* does not teach or suggest the elements missing from the combined teachings of Köster and Cantor. Accordingly, even if, arguendo, Sanghvi *et al.* teaches selectively releasable bonds containing 4,4'-dimethoxytrityl or a derivative thereof, which applicant contends Sanghvi *et al.* does not teach, the combination of the teachings of Köster and Cantor with the teachings of Sanghvi *et al.* does not teach or suggest all the elements of the claimed methods.

None of Köster, Cantor nor Sanghvi *et al.*, alone nor in any combination, teaches or suggests a method for sequencing a target nucleic acid that includes as an element identifying hybridized probes in the array by determining the molecular weight of the hybridized probes, and based on the hybridized probes, determining the sequence of the target nucleic acid. Thus, combining the teachings of Köster and Sanghvi *et al.* does not result in the instantly claimed methods of claims 71 and 72. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

Claims 38, 39, 53, 58-60, 63, 86, 88-124 and 128-144

Claims 38, 39, 53, 58-60, 63, 86, 88-124 and 128-144 are rejected under 35 U.S.C. § 103(a) over Köster (WO 94/16101) in view of Cantor (U.S. 5,503,980), because Köster allegedly teaches all elements of the claims except probes that include a double-stranded portion and a single-stranded portion, probes having 10-1,000 nucleotides or having a variable region of about 4-20 nucleotides, arrays including 10^4 or more different members or arrays of probes having sufficient sequence diversity in the variable regions to hybridize to all of a target nucleic acid molecule with complete or nearly complete discrimination, but Cantor allegedly cures these defects. This rejection is respectfully traversed.

Relevant law

See related section above.

The claims

See related section above. Claims 38, 39, 53, 89-103, 114-123 and 128 ultimately depend from claim 1 and are directed to various embodiments thereof.

Claims 86, 127 and 129 ultimately depend from claim 124. Claim 124, discussed above, is directed to an array of nucleic acid probes, where each probe includes a single-stranded portion and a constant double-stranded portion; each single-stranded portion includes a variable sequence; the array of probes has sufficient sequence diversity in the variable regions to hybridize to all of a target nucleic acid molecule; the array is attached to a solid support including a matrix chemical that facilitates the volatilization of nucleic acids for mass spectrometry; and the array includes a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between at least two nucleic acid molecules when detected by mass spectrometry. Claims 127 and 129-144 depend from claim 124 and are directed to various embodiments thereof. Claim 86 depends from claim 127, which is directed to a system including a mass spectrometer, a computer and the array of claim 124.

Teachings of the cited art and differences from the claimed methods and arrays and systems

Köster (WO 94/16101)

See related section above.

Cantor (U.S. Patent 5,503,980)

See related section above.

Analysis

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

1. The combination of the teachings of Köster with the teachings of Cantor does not result in the methods of claims 38, 39, 53, 88-110, 114-123 and 128

Claims 38, 39, 53, 55, 88-110, 114-123 and 128 ultimately depend from claim 1. As discussed above, the combination of the teachings of Köster and Cantor does not teach or suggest a method for sequencing a target nucleic acid as recited in claim 1. Neither Köster and Cantor nor the combination of their teachings teaches or suggests a method of sequencing that includes as an element identifying hybridized probes in the array by determining the molecular weight of the hybridized probes, and based on the hybridized probes, determining the sequence of the target nucleic acid.

Thus, even if Cantor teaches probes that include a double-stranded portion and a single-stranded portion, or probes having a single stranded portion of about 4-20 nucleotides, or probes having a variable region of about 4-20 nucleotides, or arrays of probes having a variable region that is determinable, the combination fails to teach or suggest detecting hybridized probes in the method of Cantor based upon molecular weight. Therefore, combining the teachings of Köster and Cantor does not teach or suggest every element of the subject matter of claim 1. Claims 38, 39, 53, 88-110, 114-123 and 128 ultimately depend from claim 1 and include every limitation thereof. Hence, the combination of the teachings of Köster and Cantor does not teach or suggest every element of the methods of claims 38, 39, 53, 88-110, 114-123 and 128. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

2. The combination of teachings of Köster with the teachings of Cantor does not result in the arrays of claims 129-132, 134 and 136-144

Claims 86, 127, 129-132, 134 and 136-144 ultimately depend from claim 124, which is directed to an array of nucleic acid probes. As discussed above, the combination of the teachings of Köster and Cantor does not teach or suggest the array as recited in claim 124. Thus, even if Cantor teaches a variety of lengths of probes, a variety of lengths of variable regions of probes and that fragments of nucleic acids comprise greater than 10^4 different members of a length between about 10 and about 1,000 nucleotides, Cantor does not teach or suggest an array or probes attached to a solid support including a matrix chemical that facilitates the volatilization of nucleic acids for mass spectrometry or an array that includes a nucleic acid probe having at least one mass-modifying functionality that increases the

discrimination between at least two nucleic acid molecules when detected by mass spectrometry.

Hence, combining the teachings of Köster and Cantor does not teach or suggest every element of the claimed array of nucleic acid probes, which includes a solid support including a matrix chemical that facilitates the volatilization of nucleic acids for mass spectrometry and a nucleic acid probe having at least one mass-modifying functionality that increases the discrimination between at least two nucleic acid molecules when detected by mass spectrometry. Therefore, the Examiner has failed to set forth a prima facie case of obviousness.

3. The combination of teachings of Köster with the teachings of Cantor does not result in the system of claim 86

Claim 86 depends from claim 127, which is directed to a system including a mass spectrometer, a computer and the array of claim 124. As discussed above, the combination of the teachings of Köster and Cantor does not result in the array of claim 124. Hence, combining the teachings of Köster and Cantor does not result in the systems of claims 86 and 127, which includes the array of claim 124.

REBUTTAL TO EXAMINER'S ARGUMENTS

1. Traverse of the Rejection of Claim 28 Under 35 U.S.C. § 103(a)

The Examiner urges that Weiss provides motivation to use the Beucage reagent. Whether or not this is correct, Weiss does not cure the deficiencies in the combined teachings of Koster and Cantor. Neither reference, singly nor in combination teaches or suggests a method of sequencing by hybridization in which hybridized probes are identified by their molecular weights. As discussed, Köster is directed to methods for Sanger sequencing in which the molecular weights of the nested fragments is determined by mass spectrometry; there is not hybridization step nor is the sequencing effected by hybridization. Köster does not teach or suggest detecting hybridized probes by mass spectrometry. Cantor, while teaching sequencing by hybridization, does not teach or suggest detection of the hybridized probes based upon molecular weight. Hence, this element of all of the method claims is missing from the teachings of Köster and Cantor. Weiss fails to provide this missing element. Therefore, the combination of teachings of these references does not result in the method of claim 28 nor of any claimed method.

2. Traverse of the Rejection of Claim 34 Under 35 U.S.C. § 103(a)

In maintaining this rejection, the Examiner alleges that Applicant's argument (apparently referring the response filed September 16, 2005) "attacked" the references individually instead of addressing the combination of the references. The Applicant respectfully disagrees. The previous response **did** address the combination of the teachings of the references and did not "attack" them individually. Attention is directed to the section at page 22 of the previous response with the header "ANALYSIS" and the header " The combination of the teachings of Köster with the teachings of Cantor does not result in the instantly claimed methods," which states:

> As discussed above, Köster does not teach or suggest a method for sequencing a target nucleic acid that includes as an element determining molecular weights of nucleic acids in the target array to identify hybridized probes, and based upon the hybridized probes, determining the sequence of the target nucleic acid. Cantor does not cure this defect. Cantor teaches arrays of probes that are partially double-stranded and partially single-stranded. There is no teaching or suggestion in Cantor to determine the molecular weights of nucleic acid fragments hybridized in a target array in order to identify hybridized probes and thereby determine the sequence of the target nucleic acid. The only teachings directed to molecular weight in Cantor are the teaching in Example 2 directed to fractionation of a sample, and reference to the Maxim and Gilbert sequencing technique, where terminally labeled DNA molecules are chemically cleaved at single base repetitions and then the molecular weight of each partially cleaved fragment is determined using electrophoresis to produce a pattern of fragments on a gel, whereby the DNA sequence can be read (see col. 1, lines 24-35). Hence, Cantor does not teach or suggest determining molecular weights of nucleic acids in the target array to identify hybridized probes, whereby the sequence of the target nucleic acid is determined. Hence, Cantor does not teach or suggest the subject matter missing from the teachings of Köster.
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> Thus, even if Cantor teaches ligating the hybridized target nucleic acids to the probes, **combining the teachings of Köster and Cantor does not result in a method for sequencing a target nucleic acid that includes as a step determining molecular weights of nucleic acids in the target array to identify hybridized probes, and based upon the hybridized probes, determining the sequence of the target nucleic acid. Hence, the combination of Köster and Cantor does not teach or suggest every element of claim 34**. Therefore, the Examiner has failed to set forth a prima facie case of obviousness. Applicant respectfully requests that the rejection be reconsidered and withdrawn. [emphasis added]

Applicant respectfully submits that the individual references were not "attacked," but instead the references were analyzed to show that the elements missing from Köster are not taught or suggested by Cantor. The teachings of the references were combined (attention is directed to the heading "the combination of teachings of the cited references" in the previous response and above in the instant response).

Furthermore, as discussed above, Köster does not teach a method for sequencing by hybridization. The disclosure in Köster referenced by the Examiner refers to a step of capturing the nested fragments for purification (conditioning) prior to mass spectrometry analysis. Hybrids are not detected by mass spectrometry, but as stated by Köster, the linkage effecting capture is designed to be temporary so that the nested fragments can "fly" off when subjected to the laser. There are no probes in the method of Köster. The sequence is based upon the determined molecular weights of the Sanger nested fragments, not by identification of any hybrids from which a sequence is deduced based upon the identity of the hybridized probes. The instant claims are not directed to Sanger sequencing; there is no step in the method that corresponds to even a single step of a Sanger sequencing method, which relies upon primers and extension thereof to produce nested fragments.

2. Traverse of the Rejection of Claims 71 and 72 Under 35 U.S.C. § 103(a)

In maintaining this rejection, the Examiner alleges that Applicant's previous argument "attacked" the references individually instead of addressing the combination of the references. The Applicant respectfully disagrees. The previous response did address the combination of the teachings of the references and did not "attack" them individually. Attention is directed to the section at page 25 of the previous response with the header "ANALYSIS" and the header "The combination of the teachings of Köster with the teachings of Sanghvi *et al.* does not result in the instantly claimed methods," which states:

> As discussed above, Köster does not teach or suggest methods for sequencing a target nucleic acid that include as an element determining the molecular weight of nucleic acids in the target array to identify hybridized probes, whereby the sequence of the target nucleic acid is determined. Sanghvi *et al.* does not cure this defect. Sanghvi *et al.* does not teach or suggest using mass spectrometry, or using mass spectrometry for sequencing nucleic acids, or hybridizing a set of nucleic acid fragments containing a sequence that corresponds to a sequence of the target nucleic acid to an array of nucleic acid probes to form a target array of nucleic acids. Sanghvi *et al.* does not teach or suggest determining molecular weights of nucleic acids in the target array to identify hybridized probes; and based upon the hybridized probes, determining the sequence of the target nucleic acid. Hence, Sanghvi *et al.* does not teach or suggest the subject matter missing from the teachings of Köster.
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> Accordingly, even if, arguendo, Sanghvi *et al.* teaches selectively releasable bonds containing 4,4'-dimethoxytrityl or a derivative thereof, which applicant contends is not taught by Sanghvi *et al.*, **the combination of Köster and Sanghvi *et al.* does not teach or suggest all the elements of the claimed methods**.
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> Neither Köster nor Sanghvi *et al.*, alone or in combination, teaches or suggests a method for sequencing a target nucleic acid that includes as an element determining molecular weights of nucleic acids in the target array to identify hybridized probes; and based upon the hybridized probes, determining the sequence of the target nucleic acid.

> Thus, **combining the teachings of Köster and Sanghvi *et al.* does not result in the instantly claimed methods of claims 71 and 72.** Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness. [emphasis added]

Applicant respectfully submits that the individual references were not "attacked," but instead the references were analyzed to show that the elements missing from Köster are not taught or suggested by Sanghvi *et al.* and that combining the teachings of Köster and Sanghvi *et al.* does not result in the claimed subject matter.

Furthermore, Sanghvi *et al.* does not cure the deficiencies in the teachings of Köster and Cantor. Sanghvi *et al.* does not teach detection or identification of hybridized probes in a method of sequencing by hybridization by molecular weight. Sequencing by hybridization methods rely upon the use of probes that include sequences covering all possible combinations. The sequence of a target molecule is deduced based upon the identity of hybridized probes. As discussed above, Köster is directed to a Sanger sequencing protocol. Mass modifying nucleotides are employed to permit sequencing of more than one fragment at time and/or to increase the resolution among the four Sanger sequencing reactions. Köster provides no teaching or suggestion for detecting hybridized probes in a method for sequencing by hybridization based upon molecular weight. Köster is not even relevant to the instantly claimed methods.

Cantor teaches using labels for identifying hybridized probes, and, in fact, teaches positional sequencing in which the locus of the label is employed in the method. Cantor does not teach or suggest *identifying* hybridized probes based upon molecular weight. Therefore, as discussed above, the combination of Köster and Cantor does not result in the instantly claimed methods which include a step of detecting the hybridized probes by their molecular weights. In such method, the sequence is deduced, not by detecting the nested fragments, but by identifying the hybridized probes.

3. Claims 38, 39, 53, 88-110, 114-123 and 128

The Examiner again urges that Applicant is arguing the references individually; this is not correct. Each reference is discussed and the deficiencies in each noted, and then the references are combined and it is shown that the combination of teachings of the references fail to teach or suggest the claimed methods. Attention is directed to the section labeled "combination of teachings of the reference" in the previous response and above. Again, as discussed above, Applicant does not dispute that Cantor teaches probes; Cantor, however, teaches detection of hybridized probes using labels, not by determining the molecular weights of the probes or by looking for a change in the molecular weight of the probes. As discussed,

Köster is directed to Sanger sequencing methods and does not describe any method in which a sequence is determined by identifying hybridized probes by molecular weight. Hence the combination of teachings of Köster and Cantor together and/or singly and/or with any of the references of record, fails to teach a method for determining the sequence of a target nucleic acid molecule by hybridization, in which the hybridized probes are detected, not by a label or any other method, but based upon their molecular weight as claimed in the instant application.

4. "Support Matrices"

The Examiner states that Cantor teaches ceramics and membranes as "support matrices" and thus teaches "a solid support comprising a matrix material that facilitates the volatilization of nucleic acids for mass spectrometry." Applicant respectfully disagrees. It appears that the Examiner is confusing the solid support with the matrix chemical that facilitates volatilization. The Examiner states on page 18 of the Office Action that:

> Ceramic and membrane matrices are claimed by Applicants in dependent claim 136 as the matrix materials are structurally identical the matrices disclosed by Cantor necessarily meet the limitation "a matrix that facilitates the volatilization of nucleic acids for mass spectrometry.

Applicant respectfully submits that, as claimed, the solid support *includes* a matrix chemical. The "matrix" referred to in claims 75 and 124 is the matrix chemical that facilitates volatilization. It is not the solid support. The specification teaches various solid supports. For example, original claim 64 specifies that the solid support is selected from the group consisting of plates, beads, microbeads, whiskers, combs, hybridization chips, membranes, single crystals, ceramics, and self-assembling monolayers. The specification teaches that the solid support can include materials, such as matrix chemicals, which assist in the volatilization process for mass spectrometric analysis (*e.g.*, see page 40, lines 3-7). The specification provides a number of exemplary matrix chemicals. For example, such matrix chemicals include nicotinic acid, 3-hydroxypicolinic acid, 2,5-dihydroxybenzoic acid, sinapinic acid, succinic acid, glycerol, urea and Tris-HCl, pH at about 7.3 (*e.g.*, see page 40, lines 3-7). Applicant respectfully submits that neither a ceramic matrix nor a membrane matrix is a matrix chemical that facilitates volatilization. In order to advance the application to allowance, claims 75 and 124 are amended herein to recite "matrix chemical" for clarity in order to more distinctly distinguish the matrix chemical from the solid support.

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In view of the above remarks and amendment, reconsideration of the grounds for rejection and allowance of the application are respectfully requested.

Respectfully submitted,

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